THE USE AND STABILITY OF MONK FRUIT PLANT-DERIVED SWEETENER IN A PROTOTYPE ORANGE JUICE BEVERAGE

By

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To my beloved family, Dr. Goodrich, Zihan and Mengzhu
for helping me accomplish this education,
discover myself and enjoy the journey

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Monk fruit (*Siraitia grosvenorii* or luo han guo) is a cucurbitaceous edible herb widely planted in China, which produces high-potency sweeteners increasingly popular in the food industry as additives in low-calorie drinks or foods. The main sweet compound, mogroside V is a cucurbitane triterpenoid saponin and the major bioactive constituent of monk fruit, which is approximately 400 times sweeter than sucrose. This study aims to clarify its applicability in juice beverages and identify its stability after thermal processing and storage. The stability of pure mogroside V in acidified model systems (pH 3.5 and 5.0) was evaluated chemically after two heat treatment regimes and during shelf-life storage over 90 days. Processing methods and storage conditions were chosen to encompass the typical shelf life of orange juice products. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to monitor the chemical degradation of the pure mogroside V compounds. Additionally, in this study, prototype orange juice beverages were developed using not-from-concentrate (NFC) orange juice and commercial monk fruit extract. Pasteurized prototypes and commercial product were compared by sensory evaluation. The results demonstrated that mogroside V was stable under all conditions tested, and a prototype formula was highly

acceptable to customers, thus indicating the potential suitability of monk fruit extracts used as a sweetener in juice beverages.

CHAPTER 1
INTRODUCTION

The growing concern with human health and the greater incidence of overweight, metabolic syndrome and diabetes have resulted in an increased interest in reduced calorie foods and beverages, especially those that use low-calorie sweeteners as sucrose substitutes (Dabelea et al., 2007). According to a very recent study, about 25% of children and more than 41% of adults in the United States reported consuming foods and beverages containing low-calorie sweeteners (LCS) in a recent nationwide nutritional survey. These numbers represent a 200% increase in consumption of LCS by children, and a 54% increase among adults from 1999 to 2012 (Sylvetsky et al., 2017). In addition, they also claimed that the LCS market is projected to continue to grow at approximately 5% per year through the year 2020.

In order to meet consumers' expectations, the food industry will likely partially or fully replace added sugar with LCS in many foods and beverages. Interest in the development of food products with LCS, and particularly natural sweeteners, has markedly increased in the last decade (Piernas et al., 2013).

As one of the natural sweeteners approved by United States Food and Drug Administration (FDA), monk fruit extract (MFE) has become more and more popular (Li et al., 2015). However, there are few scientific studies examining how MFE performs in commercial food products. This study aimed to determine the stability of monk fruit extract after thermal processing, which is one of the more common food processing unit operations, as well as the degradation rates of the main functional component over shelf-life under different storage conditions. Additionally, to demonstrate a potential application of MFE in a lower-calorie juice beverage system, we determined the feasibility of using MFE in a model orange juice beverage and developed a MFE sweetness equivalence equation with respect to by conducting a series of sensory tests with this beverage/sweetener system. This beverage case study will provide guidance for the use of MFE in lower-calorie juice-based beverages.

CHAPTER 2
REVIEW OF LITERATURE

Low-Calorie Sweetener Definitions and Applications

LCS are a class of high-intensity sweeteners which contribute no or few calories to the overall food product. They are commonly used in foods and beverages to reduce calorie content while maintaining palatability. They are also referred to as nonnutritive sweeteners, high-intensity sweeteners, and non-caloric sweeteners (Sylvetsky & Rother, 2016), depending on the specific sweetening compound.

FDA has approved several of these LCS as food ingredients, including saccharin, aspartame, acesulfame potassium (Ace-K), sucralose, neotame, advantame, steviol glycosides, and *Siraitia grosvenorii* Swingle fruit extract, commonly known as Luo Han Guo or monk fruit extract (FDA, 2014). These LCS were approved for use as table sugar, and in various food categories, such as beverages, snacks, and dairy products. However, different applications, processing methods, and food matrices of those products affect the performance of the sweetener. For example, aspartame is commonly used in soda drinks and chewing gums, but it typically isn’t used in baked goods because it loses sweetness when heated.

Most of sweeteners that widely used as sugar substitutes or sugar alternatives in commercial food products are generally called artificial sweeteners, since they are chemically synthesized. Some artificial sweeteners are derived from naturally occurring substances that have been chemically manipulated, for example sucralose from sugar; there also cannot be termed “natural”. Although these sweeteners are considered safe and potentially useful for controlling obesity-related health conditions, their use is still questioned by consumers and some research (Rodero et al., 2009; Bellon et al., 2009;

Ma et al., 2010). Therefore, natural sweeteners had gained much attention as substitutes. Studies have shown that people do not prefer artificial nonnutritive sweeteners over sucrose, especially for parents who choose products for their children (Christoph et al., 2011). Moreover, the “clean label” trend is affecting the food and soft drink category globally, leading to a backlash against ingredients perceived to be “artificial” but showing more interest in “natural” and “plant-derived” food additives and ingredients (Innova Market Insights, 2018). In terms of the use of the word “natural” on food labeling, FDA has considered that to mean the food products with nothing artificial or synthetic (including all color additives regardless of source) included in the ingredient list (FDA, 2017). Accordingly, some manufactures call their stevia and monk fruit extract sweetener “natural” because their main sweet constituents are naturally existed in plants. Currently, they are only two types of natural plant-based sweeteners which approved by FDA: certain steviol glycosides obtained from the leaves of the stevia plant (*Stevia rebaudiana/* Bertoni) and extracts obtained from *Siraitia grosvenorii* Swingle fruit, also known as Luo Han Guo or monk fruit. Both contain no calories and have sweetness-related sensory characteristics that can vary based on temperature, acidity, sweetener concentration and the chemical composition of the food product (Cardoso et al., 2004).

LCS are playing an increasing significant role for consumers who seek alternatives to sucrose in many product categories (Ng et al., 2012). The multiple characteristics of these sweeteners has also brought unprecedented challenges to new product development process. Consequently, there has been an increase in industry efforts to reduce sugar are having a profound influence on new product formation or reformulation by adjusting the sweetener level and type (Williams, 2018).

Global Food Sweetener Market Growth and Trends

Global market food sweetener sales recorded $84 billion in 2014 and is expected to increase at a compound annual growth rate (CAGR) of 4.5% and reach nearly $111 billion by 2020. Currently, in the sweetener market, sugar (sucrose) still holds the majority share (more than 80%), followed by high intensity sweeteners. Although high intensity sweeteners don't constitute the major share, this is the fastest growing segment. The market for high-intensity sweeteners is estimated to reach to $2.2 billion in 2020 at a CAGR of 5.1% (Wood, 2015). This growth reveals the rising health concerns among people and increased awareness of dietary foods.

Aspartame and sucralose are the most common sweeteners in the LCS segment (Wood, 2015). These artificial nonnutritive sweeteners have been widely used in food products and have showed beneficial influence on weight loss for children and adults (Swithers, 2013; Rogers et al., 2016; de Ruyter et al., 2012; and Tate et al., 2012). However, a major trend that must be addressed by food product developers is the public or personal pressures which are turning consumers away from artificial sugar substitutes to more natural low- or zero-calorie alternatives (Pawar et al., 2013). Accordingly, the two botanical sweeteners, stevia and MFE have gradually gained popularity in the market and enjoyed a prodigious surge in usage as natural LCS (Li et al., 2015). More specifically, it has been reported that the consumption of products that contain stevia grew by 53% from $123.1 million to $188.3 million from 2010 to 2011 in the conventional (food, drug, mass market) channels (Almendarez, 2012). In the USA, there is increased use of plant extracts known to contain highly sweet terpenoids, which result in greatly increased interest in MFE (Pawar et al., 2013). Natural LCS is seeing a major demand in the natural sweeteners category and is expected to increase more rapidly than other LCS (Hackett, 2014). This will become increasingly important as specific constituents of botanical sweeteners such as stevia and MFE are blended to take advantage of the unique attributes of individual sweet components and improve the sweet quality in the meantime (Azevedo, Schmidt, & Bolini, 2015).

Drivers of Natural Sweeteners

King (2019) noted that the number one customer driver of natural sweetener is “clean label”. A “clean label” generally means the product utilizes simple, natural and minimally processed ingredients, and possible production by traditional techniques (Edwards, 2013). Since consumers are nowadays much more interested in information about the production methods and components of the products that they eat (Asioli et al., 2017). For instance, they are concerned about the use of pesticides (Aktar, Sengupta, & Chowdhury, 2009), the use of artificial ingredients, additives or colorants (Lucová, Hojerová, Pažoureková, & Klimová, 2013), and the controversial food technologies like genetic modification (Grunert, Bredahl, & Scholderer, 2003). These factors have been encouraging consumers to look for products with healthy benefits and that are free from artificial ingredients. Accordingly, the food industry has started to respond to consumers’ demand and behavior by reformulating and supplying products that are natural and healthy (Katz & Williams, 2011). For example, in 2010, Simply Heinz ketchup was launched after removing the high fructose corn syrup from the ingredient list and replacing that with sugar (Katz & Williams, 2011).

Additionally, the FDA’s added sugar labeling policy for packaged foods and beverages could result in an increased use of low-calorie sweetener. FDA proposed the mandatory declaration of added sugars on the nutrition facts label to assist consumers in maintaining health-beneficial dietary practices (FDA 2016). Since then, the new label has begun appearing on packages on the market. The labeling requirements have been recently clarified (FDA 2019). The final guidance explains that a percent daily value for added sugars will be required on the nutrition facts label by July, 2021 to help consumers understand how their consumption will contribute to the daily sugar intake according to dietary guidelines. Interestingly, a statement of added sugars content would not be required for products that contain less than 1 gram of added sugars in a serving, making high-intensity natural sweeteners more attractive for use. Other parts of the regulation are very specific and might guide product development. For example, allulose, a monosaccharide that is naturally present in a few foods, such as wheat, figs and raisins. Williamson and others (2014) found that it was eliminated in urine without being used in human body or raising blood sugar. It was exempt from being included as a carbohydrate, sugar, or added sugar in the Nutrition Facts label on foods and beverages by FDA (2019a). Therefore, when combined with possible industry reformulations to reduce added sugar content in packaged foods and beverages, the new label policy could also drive the industry to replace conventional sugar with LCS (Huang et al., 2019).

At the same time, a combination of other factors is also driving the spread and use of natural LCS, including taxation on the sugar-sweetened beverages. For instance, Mexico implemented a 1 peso per liter excise tax on sugar-sweetened beverages in 2014, and previous studies found a 6 percent and 9.7 percent reduction in purchases of taxed beverages in the following two years, respectively (Colchero, et al., 2016).

Findings from Mexico started a trend, which had been influencing other countries to generate fiscal policies in order to take control of sugar consumption and reduce chronic disease (Colchero, et al., 2017). In the USA, Oakland, San Francisco and Seattle are among the cities that have soda taxes (Laura and Smith, 2018). As the sweeteners that serve as sugar alternative, the adoption of new technologies that reducing off-flavors (Espinoza, et al. 2014), the prevalence and cost reduction of MFE (Feng, et al. 2012), and the awareness of keto-friendly, gluten-free and hypoglycemic index compounds (Harrington, 2008; Watson, 2019) are also thought to promote the consumption of non-nutritive LCS.

Customer Perception on Products with Natural Sweeteners

Ohmes (2019) claimed that consumers want reduced-sugar beverages made with familiar ingredients but not at the expense of great taste. He believes that taste is always the single biggest driver of purchase intent. Technical recommendations from the food ingredient company Kerry affirmed these opinions in their report, “How Sweet It Is,” which looked at the sweetener taste preferences of 760 Americans across different age groups, gender and ethnicities (Kerry 2019). The results showed that 75% of tested consumers want reduced sugar products to taste the same. Moreover, 71% of American consumers note the sugar content on ingredient labels. When it comes to consumer awareness and preferences, honey, sugar (sucrose) and maple syrup were the top 3 choices at 64%, 59% and 31%, respectively. While 58% of consumers were aware of stevia, just 22% preferred it. Despite garnering 63% recognition, high-fructose corn syrup was only preferred by 7% of respondents, while aspartame ranked last in consumer preference with 6% of the vote. Moreover, according to HealthFocus International’s 2019 USA trend study, which was conducted with 2000+ respondents, the top five preferred sweeteners for US consumers are honey, fruit juices, maple syrup, agave, and stevia, while the top five "bad" sweeteners are high fructose corn syrup, artificial sweeteners, aspartame, saccharin, and fructose (HealthFocus, 2019). It's clear that consumers are educating themselves around some of these comparatively new sweetener options and understand the benefit of selecting natural sweetener over sugar or artificial sweeteners (Benn, 2002). In general, consumers perceive the term "natural" as encompassing organic production practices; they typically have a more idealized view of organic farming than what is reality (Baker, 2015). Abrams and others reported that some of the common perceptions of products labeled "natural" are that there are no preservatives, no additives, no antibiotics, no hormones, and no chemicals. Hence, the term "natural", as an information guide to customers, continues to be perceived as the safer food choice (Abrams et al., 2009). FDA (2015) has been considering clarification the term "natural" for use in food labeling.

In order to investigate customer perception more in detail, several studies have been conducted on natural nonnutritive sweeteners in different products. For example, the iso-sweetness was determined to indicate the sweet taste intensity as compared to sucrose at different concentrations. Zhang and Gruen (2013) reported the iso-sweetness of stevia, monk fruit extract, erythritol, lactitol, and xylitol to 10.1% sucrose sweetened whey protein beverages and indicated that stevia and monk fruit extract both had sweeter taste than sucrose control (77 and 115 times respectively). A sweetness intensity perception study on skim chocolate milk sweetened by stevia and monk fruit extract showed that both monk fruit extract and stevia were acceptable by young adults and children (Li, Lopetcharat, & Drake, 2015). In one study, parents preferred skim chocolate milk with natural nonnutritive sweeteners rather than that with sucrose (Li et al., 2015). However, conflicting research does exist. For example, in a sensory study conducted on functional dairy food products sensory acceptance, sucrose-sweetened aronia and elderberry kefir products were best accepted; stevia and MFE were not well accepted in either tests (Du & Myracle, 2018). Few studies dealing with juice products or acidic model systems utilizing stevia and MFE have been found in the literature. The sweetness potency, defined as the number of times sweeter a compound, on a weight basis. Stevia potency has been reported to be 202 in water and 216 in peach juice (Parpinello, Versari, Castellari, & Galassi, 2001), and 134 in mango nectar (Cadena & Bolini, 2012). Additionally, Cardello (1999) has reported that the acid pH favored the increase of the potency of stevia. In a customer preference study with sugar-reduced orange/pomegranate juice, researchers tried to investigate the effect of information about sugar reduction and the use of sweeteners on consumer perception. Results from their work showed that information about lack of added sugar and the replacement of natural sweetener influenced consumer perception of different dimensions of wellbeing. Specifically, information mainly affected consumers sensory and hedonic perception of the juice sweetened with stevia, but did not have a significant effect on MFE sweetened juice perceptions. Consumer's unfamiliarity with the sweetener might be a reason which had an impact on the results (Reis, Alcaire, Deliza, & Ares, 2017).

Monk Fruit Extract Introduction

Monk fruit sweetener or extract (MFS or MFE) is the common name for *Siraitia grosvenorii* Swingle fruit extract, a natural sweetener recently approved as GRAS by FDA (FDA 2010). Fruits of *S. grosvenorii*, known as monk fruits have a long history of use in south China as a household remedy for colds and sore throats (Kinghorn, 1986;

Prakash & Chaturvedula, 2014). In the 1970s, Lee first established that a cucurbitane-type triterpene glycoside was responsible for imparting the characteristic sweet taste of MFE (Lee, 1970). These compounds possess a triterpene backbone with two to six glucose units attached, forming mogrosides II to VI (Chang, 1996). Five chemical structures of mogroside (I, II, III, IV, and V) are recognized by the number of glucose units that are attached to the chemical structure of mogroside unit. Whereas ripe fruits of monk fruit contain primarily mogroside V and have a most sweet taste, unripe fruits, which contain less mogroside V are less sweet even bitter. Thus, the level of mogrosides sweetness depends on the percentage of mogrodside V in the total mixture of mogrosides derived from fruits (Dianpeng, et al., 2007). However, the mature status can't be distinguished by the appearance of fresh fruits. Some methods for quantitating the mogroside V in samples were reported to evaluate the quality of commercial MFE (Xia et al., 2008; Zhang et al,.2012; Sun et al., 2012). MFE works as a sweetener as combination of several different cucurbitane glycosides, mogrosides (Matsumoto, 1990),has been approved for use in dietary supplements in Japan, Australia, New Zealand, and the U.S. nevertheless (Qin et al., 2006).

It is noteworthy that none of the monk fruit extract components demonstrate toxicity to animal or humans; this is the basis for the GRAS designation by FDA. A 90-day oral toxicity study in rats by Qin et al. (2006) assessed a 30% mogroside V product containing 7.8-14.1% of unidentified components. It found no toxicity at daily doses of up to 3% of the diet, the highest dose tested. A 28-day oral toxicity study on rats by Marone et al. (2008) also confirmed the safety of MFE, which was determined to be 100,000 ppm in the diet, the highest level tested, equivalent to 7.07 and 7.48 g/kg bw/day for male and female rats, respectively. MFE was well tolerated and produced no significant adverse effects.

In addition to their sweetness properties, MFEs were reported to show demonstrated antioxidant activity. A wide range of antioxidant effects of fruit extracts, including MFE, may have anticancer, antiviral, antihyperglycemic, antidiabetic activities and some other benefits (Ukiya, 2002; Lim, 2012).

Traditional Processing and Biosynthesis Pathway of Stevia and Monk Fruit

The conventional extraction procedures of stevia leaves and monk fruit were similar. Hot-water treatment was used as a classical method (Dacome et al., 2005). And then, HPLC method was used to separate bioactive compounds and other carbohydrates by different mobile phases (Pawar, 2013). However, it should be noted these traditional processing is associated with long extraction time. Also, reproduction of stevia in the wild is mainly by seed, but germination and establishment from seed are often poor and sometimes unsuccessful (Shaffert and Chebotar, 1994). Besides, for stevia, plant organs contain different amounts of the sweet glycosides, leaves and flowers have the highest amount (Dwivedi, 1999). But, as a short-day plant, stevia flowers from January to March in the southern hemisphere and from September to December in the northern hemisphere. Accordingly, the accessibility of yield of stevia extract was hard to control. Also, for monk fruit, the mogrosides are present at about 1% in the flesh of the fruit (Kinghorn & Soejarto, 2002). But the amount is differed at different growth stages and could be heavily influenced by environmental conditions (Xia, 2008). The success of breeding depends on the choice of parents, making crosses, raising adequate population and further selections. But selection for plants producing high amounts of sweet compounds is expensive, time consuming, and relatively inefficient (Yao et al. 1999).

There was a need to develop an efficient and low-cost method that make these plant-based sweeteners utilized commercially. Adari (2015) developed a novel in situ enzymatic transglycosylation of stevioside by pre-treating the stevia leaves with cellulaseand adding soluble starch as the glucosyl donor. The results confirmed that the transglycosylation of stevioside led to an enrichment in the rebaudioside-A content from 4% to 66%. Transglucosylation of stevioside by α-amylase from *Bacillus amyloliquefaciens* in the starch solution to produce transglucosylated steviosides with reduced bitter aftertaste was also investigated (Ye et al., 2013). To date, stevia biosynthetic methods has been widely using in the industry, while mogrosides production is still based completely on extraction from fruit, which result in a high price of this products (Catani et al., 2013). The biosynthesis pathway of mogrosides has also been extensively studied recent years, and several functional genes have been identified (Dai et al., 2015; Itkin et al., 2016; Zhang et al., 2016). Dai et al. (2015) first reported their study of mogrosides synthesized in *vivo*, proposed biosynthetic pathway is shown in figure 2-2. The results also demonstrated that RNA-sequencing and digital gene expression profile analysis is a promising approach for identifying genes involved in biosynthesis of mogrosides. Mian and others (2018) successfully identified *CYP87D18* as a key *P450* gene involved in the biosynthesis of mogrosides. The *P450* supergene family is a large and diverse group of enzymes and plays critical roles in oxidative reactions in the biosynthesis of diverse natural plant metabolites (Nelson et al., 1996). In the yeast *BY4741-Z5* expressing *SgCbQ/CYP87D18/CPR*, three intermediate products were detected between cucurbitadienol and mogrol. In the future, further characterization of other related genes involved in the biosynthesis of mogrosides may permit engineering of recombinant yeast that produce mogrosides with high yield.

Metabolism and Biotransformation of Mogrosides

Researchers believed that the glycosidic bonds are not easily broken by either human digestive degradation or the action of intestinal microorganisms, which indicates that the product has neither caloric nor glycemic properties (Suzuki et al., 2005). Mogrosides are therefore non-nutritive constituents whose sweetness intensity are 100 to 250 times sweeter than sucrose (FDA, 2014). There are a few studies on the metabolism of mogrosides. One important study (for potential use by the food industry) investigated the biotransformation of mogroside III by human intestinal microflora *in vitro*, which suggested that human intestinal bacteria showed potent ability to transform mogroside III to release secondary glycoside mogroside II and the aglycone mogrol (Yang et al. 2007); a recent study illustrated that the MFE was stable in simulated gastric and intestinal juices *in vitro*, as only several intact mogrosides were detected during incubation in model digestion systems (Guisheng et al. 2017). However, Murata (2010) did a research on the *in vivo* digestion, absorption and metabolism of mogroside V in rats, whose results indicated that dehydrogenation, deoxidation, oxidation and isomerization were the major metabolic transformations of mogroside V, these results were contrary to previous reports. Then, the metabolites were found to be different in the biotransformation of MFE in normal and type 2 diabetic patients in this study. This was consistent with another study in rats, where diabetic model rats produced more metabolites than healthy rats. With respect to distribution studies, there was a study investigating the digestion and absorption of mogroside V in rats (Zhou, Zhang, Li, Wang, & Li, 2018). Researchers found that mogroside V could be degraded by digestive enzymes and intestinal microflora and was excreted in the feces as mogrol and other metabolites. The total amount of mogrosides in the feces was about 61% of administered amount; however no mogroside V was detected in the whole blood or urine and therefore the absorbed amount of mogroside V and its metabolites was also extremely low. Reseachers claimed that the mogroside V is mostly excreted without absorption in rats. Similar results were presented in another study as well (Xu et al., 2015). After grounded monk fruit administration to rats, mogroside V was the most abundant compound in GI tract of rats. The other metabolisms were mainly excreted by feces, while mogroside V was mainly excreted by urine as the original structure.

GRAS Notifications and Manufacturing Process of Monk Fruit Extract

In July 2009, FDA received a GRAS notice from BioVittoria (Hamilton, New Zealand). The subject of the notice was *Siraitia grosvenorii* Swingle (Luo Han Guo) fruit extract (SGFE). With mogrosides V constituting more than 30% of their liquid and powdered product, BioVittoria received a “no question at that time” response from FDA in January 2010 (FDA, 2010). This response indicates the ingredient can be used unless there are adverse effects reported by either the marketplace or in the scientific literature. Since then, monk fruit extract started to be used as a sweetener and flavor enhancer in foods, as well as use as a tabletop sweetener since then.

Based on the primary evidence of safety provided in the first GRAS notice and additional studies, Guilin Layn Natural Ingredients received a “no questions at this time” response from the FDA in April 2011. The product they provided was a monk fruit juice concentrate with a soluble solids level of 65° Brix based on the concentration of mogroside V, which was intended for use in conventional foods as well as infant and toddler foods.

In January 2018, Nutramax's Luo Han Guo fruit extract powders also received the same response from FDA. Those powders were manufactured in a process similar to those described in previous GRNs but with more purification steps, the company produced highly purified products (up to 95% mogroside V) using current Good Manufacturing Practices (cGMP).

So far, this substance has been used as a sugar substitute in different foods at levels proportional to those specified in five different GRAS notices (GRNs 301, 359, 522, 556, and 706).

In general, the commercial preparations (extracts) described in the GRAS notifications discussed above were obtained by mechanically crushing or shredding the fruit, which was then extracted with hot water. Centrifugation and ultrafiltration were then used to remove protein and pectin to produce a liquid fruit concentrate. Activated carbon and/or adsorption/separation polymer resin columns were used to further purify the ingredient(s) of interest by absorbing glycosides onto their surface(s). The desired components were then washed from the resin with ethanol, which could then be removed by evaporation. Extracts as described in the two GRAS notifications are now commercially available (Pawar et al., 2013).

Orange Juices and Orange Juice Beverages

In the United States, orange juice is the most popular juice per capita, leading juice consumption at 10.2 liters in 2015 (USDA, ERS. 2015). Meanwhile, the interest in developing functional food is rising, driven largely by the market potential for foods and beverages that can improve the health and well-being of consumers (Hilliam, 2000).

Experiments (da Costa et al., 2017; Luckow & Delahunty, 2004) have shown that a population of consumers significantly preferred the functional orange juices. This indicates that a potential market for functional orange juices does exist (Luckow & Delahunty, 2004). Furthermore, orange juice is a candidate for application of sweeteners studies since its fresh flavor characteristics are favored by customers (Liu, 2003) but many consumers are seeking that flavor in a lower calorie product. In addition, because these products are generally preserved by thermal processing (Jimenez-Sanchez et al., 2017), this is an appropriate application for a potential monk fruit sweetened product. However, it should be clearly defined the differences between "orange juice" and "orange juice beverages/products". Many countries have several regulations governing fruit juices. Generally, only beverages that are 100% juice can be called "juice". Otherwise, according to most of regulations, the beverages that are diluted to less than 100% juice or added other additives must have the word "juice" qualified with a term such as "beverage," "drink," or "cocktail" (FDA, 21 CFR 102.33). Accordingly, in the European Union and many other countries, the term orange juice may only be used for juice extracted from sweet oranges, *Citrus sinensis*. In the US, however, regulations allow for up to 10% of tangerine or hybrid orange/ tangerine juice to be included in orange juice. Codex standards, a collection of internationally recognized standards relating to foods, permit the inclusion of 10% mandarin juices as well. These added mandarin-type juices are thought to improve the color and flavor of blended juice.

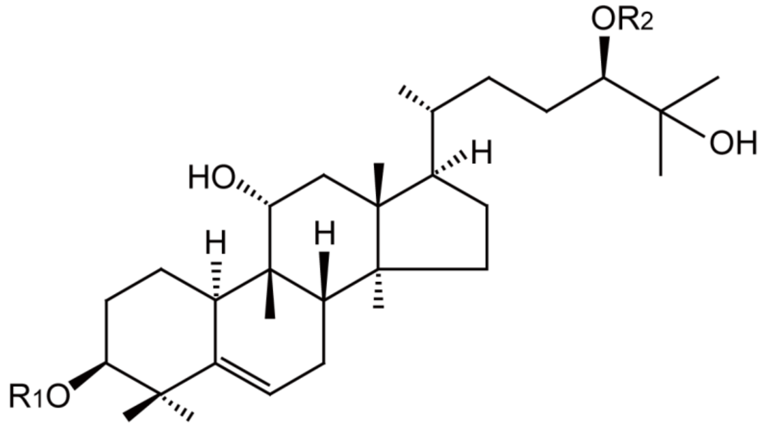
Important Quality Parameters of Orange Juice Beverages

USDA (1983) issues the grade standards for fruit juices. Grade standards concern product quality. The terms "Fancy" or "Grade A" may only be used on products meeting all the specifications defined for such grades by the USDA. For instance, some of the requirements that orange juice produced in Florida to be labelled as Grade A must meet are shown in Table 2-1(Fellers, 1990). Analytical parameters can be determined by standard methods of analysis to give reliable results. The quality factors are measured on a 100-point scale. For example, orange juice flavor is evaluated by sensory means; As for color specification, juice manufactures would perform visual comparisons of orange juice in a standard glass tube to set of USDA plastic comparators also in glass tubes (Lee, 2000).

The most important properties of orange juice are its sugar content and ratio of sugar to acid (Fellers, 1990). Brix degree for orange juice, normally represents the sweetness of products, not only includes the concentration of dissolved sugars but all soluble solids. The level of acid is often measured to indicate the acidity in the juice. Thus, this ratio is an important indicator for taste. As orange ripen, the ratio increases as sugars are formed and the acid content decreases (Fellers, 1990).

Orange Production and the Orange Juice Market

Currently, citrus producers in many countries are facing serious citrus production problems (Spreen and Zansler, 2016). For instance, as the world's largest orange producer, Brazilian commercial orange production continued decreasing due to high temperatures and stress from the precious production cycle (USDA 2018). At the same time, China's production is projected down slightly on unfavorable weather, resulting in a smaller crop in several provinces (USDA, 2019). Huanglingbing (HLB) or citrus greening was responsible for the decrease in the production of citrus in the United States from 7.98 to 2.22 billion tons (72.2% reduction) from 2007-08 to 2017-18 (USDA 2018). Additionally, U.S. orange production was decreased due to other reasons including the damage by Hurricane Irma in 2017 as well as the recent hot weather in California (USDA 2017). However, the fresh fruit market was less impacted than the juice industry, as the juice industry relies on large quantities of fruit for processing efficiencies. Around 90% of the oranges produced in Florida are used for producing orange juice, and it's difficult to find fruit not showing HLB symptoms in this state (Dala-Paula et al., 2019). Singerman et al. (2017) reported an increase from $2.89 to $9.34 (3.2 times) of the price of a box of orange since HLB had been detected and widespread in the United States, presumably due to lack of supply. However, the control of HLB is still difficult; one of current strategies focus on vector control and management of infected trees (Batool et al., 2018), which are theoretically possible and effective. However, the bacteria species associated with HLB are mainly transmitted by insects, namely psyllids (Bové, 2006). Methods developed to prevent contact between the pest and trees are costly and time-consuming (Ferrarezi et al., 2017b). The most effective control strategy has been to remove infected trees in a growing area, but it is uncertain how long a tree can be infected before showing the symptoms of the disease (McCollum and Baldwin, 2017). Florida growers have been using foliar nutritional spray products to compensate for lack of nutrient by the disease. Unfortunately, the beneficial effect of this approach may not manifest unless the vector is thoroughly controlled, since the trees are still infectable (Gottwald et al., 2012; Plotto et al., 2017).



	R1	R2
Mogrol	-H	-H
Mogroside IE	-Glc	-H
Mogroside IIE	-Glc	-Glc
Mogroside IIIE	-Glc	-Glc^{2}-Glc
Mogroside IVE	-Glc^{6}-Glc	-Glc^{2}-Glc
Mogroside V	-Glc^{6}-Glc	-Glc^{2}-Glc ^{6}\Glc

Figure 2-1. Structures of main mogrosides isolated from the fruits of monk fruit

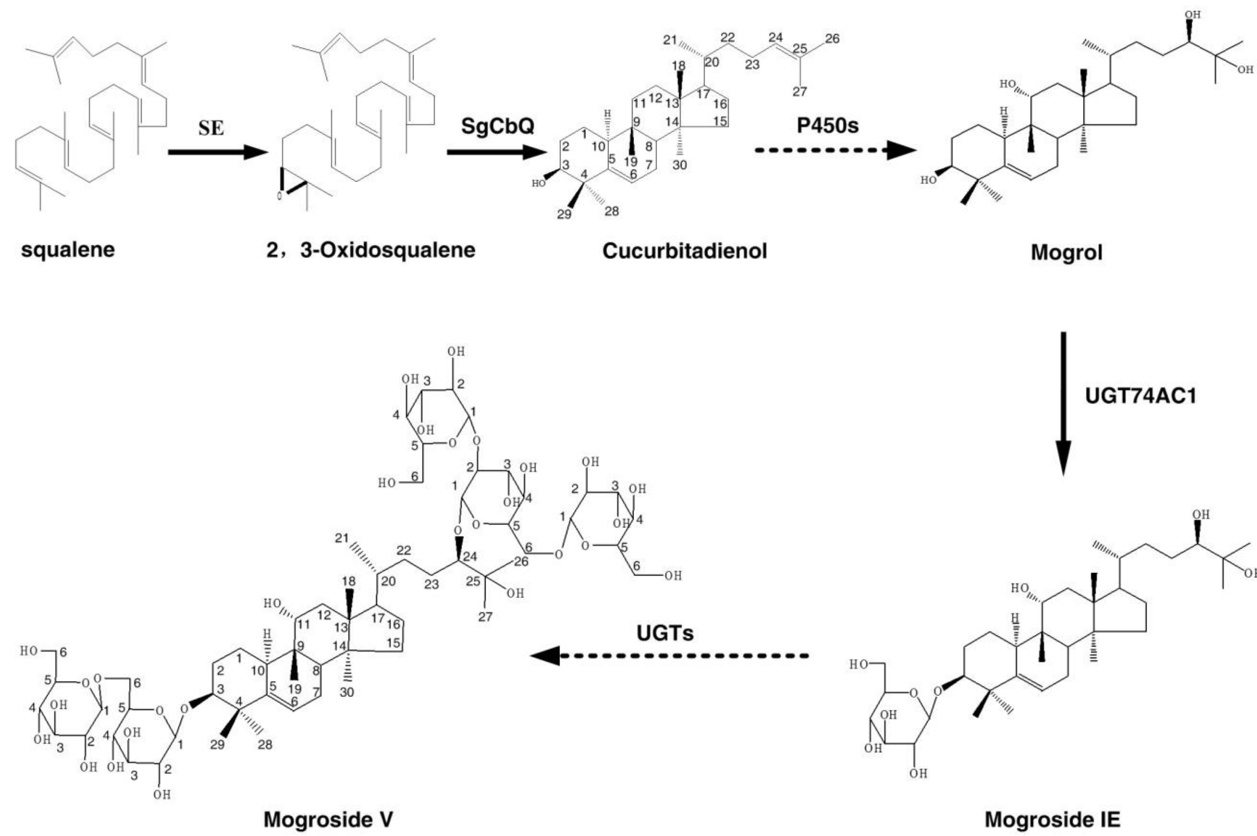


Figure 2-2. Proposed biosynthetic pathway of mogrosides in monk fruit (adapted from Dai et al. 2015): SE, squalene epoxidase; SgCbQ, cucurbitadienol synthase; P450s, cytochromes P450; UGTs, uridine diphosphate glycosyltransferases. Dark solid arrows represent one step; dark dotted arrows represent multiple steps.

Table 2-1. USDA standards for Grade A orange juice products from Florida[a]

Standard Parameters	Orange Juice (Not from concentrate)/ Processed Orange Juice Products/ Pasteurized Orange Juice
Analytical factors	
°Brix (% w/w)	Min. 11.0
°Brix to acid ratio	12.5-20.5
Quality factors	
Appearance	Fresh orange juice
Color	Very good, 36-40 points
Flavor	Very good, 36-40 points
Minimum score[b]	90

[a] Adapted from Fellers, 1990.
[b] A limiting rule applies in which the lowest score of any one factor determines the grade.

CHAPTER 3
MATERIALS AND METHODS

Retail Screening of Commercial Low-calorie Orange Juice Beverages

Low-calorie orange juice beverage products found at the retail market were evaluated for formula determination. The commercial low calorie OJB included Trop50® No Pulp (Tropicana Products, Inc., Chicago, IL), and Minute Maid® Pure Squeezed Light No Pulp Orange Juice (The Minute Maid Company, Sugar Land, TX). These were analyzed for standard quality parameters and sensory sweetness perception. The nutrition facts of these commercial products were also summarized for formula adjustment.

Beverage Analysis

Total Solids (°Brix) Measurement

Soluble solids (°Brix) were measured using a hand refractometer (0-32% Brix, Fisher Scientific, Pittsburgh, PA, USA). Refractive index was recorded and converted to °Brix. Measurements were performed at 20.0 ± 0.5 °C. The refractometer prism was cleaned with distilled water after each analysis.

pH Determination

The pH of treated beverages and untreated orange juice samples was measured using a digital pH-meter (Accumet AB150 pH Benchtop Meters, Fisher Scientific, Pittsburgh, PA, USA). The meter was calibrated with commercial buffer solutions at pH 7.0, 4.0 and 2.0. Samples (10 mL) were placed in a 50 mL beaker with a magnetic stirrer and pH electrode inserted. Samples were measured at 20 ± 0.5 °C.

Titratable Acidity (TA)

Samples of 20 mL were placed into a 250 mL beaker, and 80 mL of distilled water was added. This solution was then titrated against standardized 0.1 N NaOH (Sigma-Aldrich, Dublin, Ireland) to the phenolphthalein end point (pH 8.2 ± 0.1). The volume of NaOH was converted to grams of citric acid per 100 mL of juice, and TA was calculated.

Color Determination

Samples were cooled to room temperature (20 ± 1.0 °C). Color was measured using a Hunter Laboratory colorimeter (ColorQuest XE, Hunter Associates Laboratory Inc., Reston, VA) based on three color coordinates, namely, L*, a*, and b*. The instrument was calibrated using white and black reference tiles. Color values were measured using total transmission mode.

Viscosity Determination

The viscometer (RVDV-II+P, Brookfield Engineering Laboratories, MA, USA) drives a spindle immersed in 500mL test fluid. When the spindle is rotated, the viscous drag of the fluid against the spindle is measured by the deflection of the calibrated spring. The spindle type and speed combinations will produce satisfactory results when the applied torque is between 10% and 100%; Spindle S01 was used in this experiment. And viscosity of each samples was determined at 100 RPM spindle speed.

Mogroside V Stability in Model Juice Systems

Two citric acid-added model systems (pH 3.5 and 5.0) were prepared into which pure mogroside V was dissolved at 25mg/100mL. The heating experiments were carried out at 72 °C for 15s and at 90 °C for 30s, as commonly used thermal processing methods in juice industry. Samples of the sweetened model juices with and without heating treatments were kept in tightly closed glass tubes and stored in the refrigerator at 4°C. The samples were chemically monitored periodically over shelf life for 90 days. LC-MS/MS were used to analyze compound losses and degradation products.

Model Juice Preparation

A representative model juice was formulated and prepared according to the screening of purchasable products at the retail market. This study mainly focused on the stability of main ingredient, mogroside V (98%, from *Siraitia grosvenorii* (Swingle), Frontier Scientific, UT, USA) in acidic liquid after thermal processing and over shelf life. Thus, distilled water was used as a simple basement in model juice. Sodium citrate (Fisher Scientific, NJ, USA) and citric acid (Fisher Chemical, NJ, USA) were also added to reach the desire pH 5.0 and 3.5, which represented the pH range of many juice products. In addition, 6.0g sucrose was added to replicate the soluble solids content of commercial products, and mogroside V was added and tasted to achieve the equivalent sweetness in comparison to the commercial products as a reference. Model juice of different pH levels were mixed separately and pooled into 3 groups of 10 mL tubes: untreated juice (Control), Treatment 1, and Treatment 2.

Thermal Processing Treatment on Model Juice

Pasteurization methods applied on model juice were selected with reference to industrial practice where heating of orange juice is carried out (Braddock, 1999). Treatment 1 represented a conventional mild temperature-short time (MTST) thermal processing at 72 °C for 15s (Walkling-Ribeiro et.al. 2010). Treatment 2 represented a typical high temperature-short time (HTST) thermal treatment of orange juice at 90°C for 30s (Aguilar-Rosas et al 2013). Both methods met the requirements of FDA and could result in a population reduction of at least 5-log in *Salmonella*, as well as other potential pathogens in orange juice (Petruzzi et al. 2017).

A water bath (Precision GP 05, Thermo Fisher Scientific, Waltham, MA) was used for thermal treatments. After the target temperature reached, 15 mL glass tubes with 10 mL of model juice, closed with plastic caps, were submerged into heated water bath. The liquid level in the test tubes were below the water level. An additional tube with thermometer was added to measure and indicate the temperature. Tubes were removed and immediately cooled in an ice bath right after the holding time for each treatment to mimic the industrial practices.

Shelf Life Study and Stability Test of Pure Mogroside V

A 90-day shelf life storage test for the model juices was carried out at 4 °C. Samples were kept in tightly closed glass tubes during shelf life. The model juices were chemically monitored periodically. LC-MS/MS were used to analyze mogroside V losses every 30 days. The effect of heat treatments was evaluated by comparison the mogroside V content of non-treated samples and samples right after thermal treatments. Stability of mogroside V at different pH at were also determined.

Preparation of Standard Solutions

Stock solution of mogroside V was prepared by dissolving the mogroside V powder in water at a concentration of 1.0 mg/ml. Additionally, stevioside was selected and used as an internal standard in this study due to its similar molecular weight to mogroside V. Stock solution of stevioside was also prepared in water at a concentration of 1.0mg/ml. A mixed solution was diluted with water to obtain a series of standard solutions with concentration of 0.25, 0.5, 1, 2, 5, and 10 mg/L. Linearity of response was determined for six concentrations. The calibration curve was based on the relationship of mogroside V concentration to peak area.

Sample Preparation

Accurately measured 10 µL model juice from each glass tube and 10 µL of internal standard, stevioside solution (0.2 mg/mL) were introduced into a 2mL centrifuge tube and diluted with 1 mL water. The mixture was vortexed for 30 seconds to ensure the ingredients were dissolved. Then, 70µL of the formed sample were transferred into a screw neck vial. A 10 µL solution was injected for LC-MS/MS analysis.

LC-MS/MS Analysis

The liquid chromatography (LC) separation was performed using an Ultimate 3000 system (Dionex, Sunnyvale, CA, USA), equipped with a rapid separation (RS) pump, an RS column compartment and an XRS open autosampler. A ACQUITY UPLC BEH C18 column (150 × 2.1mm, 1.7µm particle size) at a column temperature of 35°C. The mobile phase consisted of water (solvent C) and acetonitrile (solvent D) with gradient elution as follows: 0-5 min, 90% solvent C; 5-10min, 95% solvent D; 10-15min, 90% solvent C. The flow rate was set to 0.2 mL/min and the injection volume was 10 µL.

The mass spectrometry (MS) was carried out using a triple quadrupole mass spectrometer (TSQ Quantiva, Thermo Fisher Scientific, San Jose, CA, USA) with selected reaction monitoring (SRM). The instrument was operated with a heated-electrospray ionization (HESI) in positive/negative ion mode. The ion source conditions were set as follows: positive ion, 3500V; negative ion, 2500V; sheath gas, 35 Arb; aux gas, 10 Arb; sweep gas; 0 Arb; ion transfer tube temperature, 325°C; and vaporizer temperature, 275°C. The MS/MS parameters were optimized as follows: collision gas pressure, 2mTorr; source fragmentation voltage, 0 V; chrom filter, 0 s; and dwell time, 200 ms. The SRM transitions, collision energies and RF lens of the analytes and internal standard are shown in Table 3-1. Xcalibur software (Ver. 3.0) was used for data processing and instrument control.

Sensory Evaluation of Prototype Orange Juice Beverages

Initial formula was determined using conventional orange juice as a reference. After formulation, bench scale products were prepared for preliminary test, which showed the perception of MFE and provide some guidance for final formula determination. Then, samples were reformulated and adjusted based on consumers' feedback in preliminary test. Prototype beverages were evaluated by panelists in final sensory evaluation. The aim of this phase was to investigate consumer hedonic and sensory perception of prototype orange juice beverages with MFE. Meanwhile, the sensory differences of stevia and MFE as a sweetener used in orange juice beverages were also determined. The effects of information about sugar reduction and the use of sweeteners were indicated.

Preliminary Test on Bench Scale Products

The method of the preliminary test involved in two basic steps: formulation determination and sensory test. In order to prepare low-calorie orange juice beverage comparable to commercial Trop50®, conventional Tropicana orange juice, purified water, food grade citric acid, and commercial monk fruit extract sweeteners were used for formulation. All formulated samples were prepared by following method: 50% Tropicana orange juice (v/v), 50% water (v/v), and then monk fruit extract (Monk Fruit in the Row, Cumberland Packing Corp. NY, USA) and citric acid (Now Foods, IL, USA) were added to replicate the taste of conventional orange juice. Tropicana orange juice with no pulp was used as flavor reference to determine the best formulation. Brix value, pH and titratable acidity were measured to assist the formula determination and indicate the sweetness, sourness, and balance of sweetness with sourness of tested formulation (Parish, 1998). The dextrose existed in the commercial monk fruit extract product was aimed to make the products measurable for customers using, but it could influence the Brix degree. Accordingly, the amount of sweetener and citric acid used in each product was adjusted and determined by tasting. Formula details are shown in appendix B.

In the preliminary sensory test, 31 panelists were selected randomly on campus, in which there were 12 male panelists, 19 female panelists, to conduct the a single-blind taste panel on randomized-coded juice samples. Tropicana orange juice with no pulp (OJ-Original), Trop50®, and the formulated orange juice beverage (FOJB) were evaluated with 12 attributes with respect to appearance, texture, flavor, and aftertaste of juice beverages. Overall appearance, color, aroma, overall flavor, and overall freshness were evaluated based on 9-hedonic scale. Flavor and mouthfeel attributes including sweetness, sourness, thickness, and tartness were evaluated with 5-anchored scale, in which situation, rating of 3 represented “just about the right”. The aftertaste of each sample was also evaluated to indicate the lingering effect resulting from juice beverages. Ultimately, panelists were asked to rank the samples from 1 to 3 to indicate their preference on the 3 juice beverages. They were also asked to rate their potential to purchase orange juice beverage with claims of “low-calorie, natural sweetener” on a 5-hedonic scale. Two-way ANOVA without replication and Friedman’s analysis were used for data analysis.

Pilot Plant Scale Products Manufacture

Based results derived from the preliminary test, two different formulas of low-calorie orange juice beverages were developed. The formulas of sample A and B were the same formulation but with different MFE sweetener concentration based on results and comments from the preliminary test. Sample A has the same sweetness level with regular orange juice based on the result of sweetness equivalent test. However, considering reducing the potential bitter taste of sugar substitute and saving cost, sample B was prepared with 25% less amount of MFE sweetener added. Additionally, natural color additives (“Spicy Yellow” and “Mandarin”, Exberry, NY, USA) were also added to the all final samples to create similar appearance to commercial products.

After the addition of all ingredients and mixing, the orange juice mixtures A and B were pasteurized individually in a pilot scale pasteurizer with a tubular heat exchanger (UHT/HTSTLab-25EHVH, MicroThermics, NC, USA) at 90 °C for 30 s. Details of the thermal treatment are described: samples flow rate was maintained at 1L/min, which was shown on the flow meter; products were preheated to 60°C, and then continuously heated until the temperature reached 90 °C; products were held in the holding tube with the temperature maintained at 90 °C for 30 s; the cooling tank equipped in the system was used to make the product temperature was controlled to 40°C; the pasteurized and cooled juice beverages were aseptically collected into aseptic containers with sealed lids and stored at 4 °C for 36 hours until final sensory evaluation.

Final Sensory Evaluation - Customer Preference Test

Final sensory test was performed in the Taste Panel at the University of Florida, in a separate booth area to enable participants to conduct the task comfortable and without distractions.

Pasteurized sample A, sample B and commercial product Trop50® were evaluated by sensory test. Commercial low-calorie orange juice beverage Trop50® contains stevia as sweetener to maintain the sweetness was used as a comparison to provide reference for the use of MFE in lower-calorie juice-based beverages. Tests were conducted in the morning, three samples (2oz each) were served in clear plastic cups, received random 3-digit codes and they were presented to the consumers at one time but in a randomized sequence.

92 panelists were recruited based on orange juice consumption and acceptance of low-calorie beverages. Demographic information was collected, such as age and gender. Consumer familiarity with juice products and light beverages was assessed. Then, they were asked to rate the overall appearance, overall liking of each sample using 9-point scale (1=Dislike extremely, 5=Neither like nor dislike, 9=Like extremely). The color, sweetness, sourness, bitterness, and thickness of each sample were also asked by using just-about-right 5-anchored hedonic scale (e.g. 1= Not nearly sweet enough, 2=Somewhat not sweet enough, 3=Just about right, 4=Somewhat too sweet, 5=Much too sweet) to investigate the optimum levels of these attributes in tested products. Check-all-that-apply questions composed of 11 sensory terms: Smooth, Delicious, Lingering, Refreshing, Fresh, Chemical, Fruity, Natural, Artificial, Thin/watery, and Other. These sensory terms were selected based on former studies (Reis, Alcaire, Deliza, & Ares, 2017) in which consumers were asked to describe low-calorie juice products. Panelists were asked to rank all samples and indicate their purchase intents on each sample. Consumer attitude toward purchasing products was asked again after product ingredient and nutrition information was given. Data were collected using Compusense software (Compusense Inc., Guelph, Ontario, Canada). Ballots shown in the appendix.

Statistical Analysis

Data are shown as mean ± standard error (SE) of triplicate measurements. Sensory data was analyzed with analysis of variance (ANOVA) using Compusense software (Compusense Inc., Guelph, Ontario, Canada). Tukey's Honest Significant Difference (HSD) test was used for mean comparisons as well. Statistical analysis on instrumental data was carried out using ANOVA by GraphPad software (GraphPad Software, CA, USA).

Table 3-1. SRM transitions, collision energies and RF lens for LC-MS/MS analysis of samples

Compound	Retention Time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)	RF lens (V)
Mogroside V	4.8	1285.64	1123.56	55	221
Stevioside	5.3	803.34	641.23	19.05	101

CHAPTER 4
RESULTS AND DISCUSSION

Stability Studies on Mogroside V in Model Juices

Standard Curve of Mogroside V

As LC-MS/MS was used for compound analysis, linearity of detector response was determined in the concentration range 0.25-10 mg/L of mogroside V using 6 standard solutions. A linear calibration curve was obtained with a correlation coefficient of 0.9995 (Fig. 4-1).

Heat and pH Stability

In citric acidified aqueous solution mogroside V was remarkably stable after thermal processing (Fig. 4-2). The mogroside V calculated amounts represent mean values from triplicate analyses. Control values indicate the amount of mogroside V in the model liquid without heat treatment at the same time as other treatments. In each pH group, thermal processing did not significantly ($p \leq 0.05$) affect the levels of mogroside V. Aqueous solutions containing mogroside V are reported to be stable under boiling conditions (Nabors and Gelardi, 1986). Additionally, different pH level also didn't cause any significant effect before and after thermal treatments. Although there are no literature reports detailing the stability of mogroside V. It is occurring in fruit, which is usually a low pH environment, and the indigenous use of monk fruit involves drying, boiling, indicates that these bioactive compounds are likely to be a heat and pH stable molecule. In addition, structurally, Lindley (2012) believed that mogrosides should be stable since they resemble the steviol glycosides that are known to exhibit excellent stability. Early work by Lee (1975) also showed that the β-linkages of glucose units ensure that the mogroside V is a stable compound and intrinsically resistant to hydrolysis. Overall, in no study was there a significant difference detected under thermal treatments in either pH 3.5 or 5 products, indicating pH and heat stability of the compound of interest, mogroside V. Also, this result demonstrated that there had been no loss of sweetener nor any interaction with citric acid in the acidic model juice system under commonly used thermal treatment 1 (72°C, 15s) and treatment 2 (90°C, 30s).

Shelf Life Study/Storage Stability

The results from the model juice storage trials are documented in Fig. 4-3. The amounts of mogroside V also represent mean values from triplicate analyses. According to data analysis, pH 3.5 and pH 5.0 acid conditions did not result in any significant ($p \leq 0.05$) degradation of mogroside V during 90-day storage at 4°C. Moreover, the four groups of pasteurized model liquid stored after 90 days did not show any significant ($p \leq 0.05$) change in their mogroside V levels. The results from repeated experiments were shown in Fig. 4-4. Thus, the fact that no significant ($p \leq 0.05$) differences was detected for 90 days shelf life for both studies suggest that mogroside V does not degrade in typical juice beverage applications. The stability of mogrosides has been investigated in baked mince pork slices as natural antioxidants during the storage by Cheng and others (2017), their results showed that after baking process (180°C for 3min), more than 75 percent mogrosides could be retrieved for all groups after the storage, thus, they claimed that mogrosides were relatively stable during storage for 21 days at room temperature in pork products. The conclusion was consistent with the analytical results of this study that pH, storage time, and the interaction of pH and storage time do not have any significant degradation regarding the initial mogroside V levels. Concentrations were maintained at the same level indicating that mogroside V in acidic liquids stored at 4°C was stable, even after 90 days storage.

Sensory Evaluations on Application of Monk Fruit Extract in Prototype Orange Juice Beverages

Determination of Optimal Formula Sweetened with Monk Fruit Extract

According to the lab scale sensory test by a small group of panelists, the optimal formulation for orange juice beverage was determined, details are shown in appendix B. That was 0.2% citric acid and 1.6% monk fruit extract (w/v), adding to orange juice mix with purified water in 1:1 ratio. Mogroside V has been rated as being in the rages 250-425 sweeter than sucrose by human taste panel, depending on the concentrations of the tested samples (Kinghorn et al., 1998). The optimal formula in this study was selected by comparing the sweetness and sourness with reference original orange juice samples. Formulated sample tasted most similar to original orange juice with an acceptable pH level and Brix degree, thus it was brought up to upcoming preliminary sensory analysis to be evaluated. The pH, degrees Brix and acidity of the sensory products is shown in Table 4-1. Based on the result, formulated beverage showed a lower pH degree and acidity, which indicated the taste of formulated OJB might be sourer than other samples. Also, the Brix level was slightly higher than the Trop50® commercial product. The pure monk fruit extract had no effect on the Brix level. However, the reason for the increased Brix degree was the dextrose existed in the commercial monk fruit extract product as discussed above. Dextrose, as know as glucose, is a simple sugar derived from corn. The relative sweetness value for dextrose is 74 reported by Biester, Wood and Wahlin (1925). According to the product introduction, dextrose was added to many sugar substitutes that in powder form to make it measurable for consumers. Thus, the main sweetening ingredient in Monk Fruit In the raw is simply monk fruit.

Preliminary Sensory Test: Comparison of Commercial Products and Tested Formulation

After preliminary development, the formulated sample, conventional orange juice and Trop50® were prepared and evaluated by 31 panelists in the preliminary test. Before tasting, panelists were asked to observe the samples only to evaluate the appearance, color and aroma. As shown in Table 4-2, the overall appearance and aroma of original orange juice and Trop50® had no significant difference, but significantly higher rated than that of formulated OJB. It was reasonable due to the dilution of color with the addition of water in the formulation. The aroma and overall flavor of original orange juice was significantly higher rated than that of Trop50® and formulated OJB, which indirectly verified that the higher concentration and abundance of flavor-active compounds in orange juice played an important role for rich flavor and pleasant aroma of orange juice. With reduction of orange juice compensated with water, it was difficult to maintain the similar flavor profile as it before dilution. However, it was notable that rating of overall freshness of FOJB was significantly higher than that of Trop50®, which may indicate that compare to Trop50®, the overall acceptability of OJB with MFE was better received by participants.

The results shown in Table 4-3. was observed that the formulated sample was rated with significantly higher sweetness and significantly lower tartness than the other two products, while the thickness rating was significantly lower than the other two products. The sourness level of formulated OJB and Trop50® was similar, but significantly lower than the sourness of Tropicana Orange Juice. Results of sweetness and sourness complied with the results obtained from chemical analysis as shown in Table 4-1. The formulated OJB with lower acidity but higher Brix value explained the sourness and sweetness ratings in sensory analysis. According to the ingredient list of Trop50® (Appendix A), gellan gum was used as an ingredient, presumably as an emulsifier and thicken agents to increase the mouthfeel of Trop50®, making it comparable to real orange juice. Additionally, the lower thickness and tartness ratings of sample were also partially explained by the same reason for ratings of color and flavor, which was the decrease in characteristic compounds resulting from dilution of original juice formulation. Philipsen and others (1995) also reported that color had specific unique effects on overall acceptance, flavor quality, and intensity in sensory responses to a flavored cherry beverage. There have been mixed results on how color influences the flavor intensity. Kostyla (1978) reported that lower scores were received when yellow color was added to raspberry beverages. However, Dubose et al. (1980) and Johnson and Clydesdale (1982) showed that the flavor ratings of beverages increased as the color intensity increasing.

The results of the aftertaste analyses of three samples are shown in Fig. 4-5. Compared with the results from original orange juice, evaluation on aftertaste of Trop50® and formulated OJB were similar but still slightly different. Formulated sample had more panelists (23%) rated with favorable aftertaste than Trop50® (13%). Over half of panelists (52%) believed there was non-favorable aftertaste in Trop50®. This indicated that the acceptability of the OJB sweeten with MFE in this study might higher than stevia, which also explained that the unfavorable aftertaste of sweetener may be a reason for the low hedonic scores of the Trop50® in the flavor attribute. Guggisberg and others (2010) reported bitter and off-flavors in whole milk yogurt sweetened with stevia alone had negative influences on physical and sensory properties. Accordingly, dislike of stevia sweetener might also have influenced the preference ratings for formulated OJB, which tended to be higher ($p \leq 0.1$) than ratings on Trop50®. It was a good indicator for the possible success of the final prototype OJB sweetened by MFE in the pilot plant scale sensory evaluation.

Final Sensory Evaluation: Customer Preference Test on Sweetener in Low-calorie Orange Juice Beverages

Sample A and Sample B were formulated based on the results from preliminary test and instrumental analysis. Color additives (0.15% shade "Spicy Yellow" and 0.014% shade "Mandarin", w/v) were added to both formulas in order to adjust the appearance, making the color looks like fresh orange juice. The beverage analysis results of final samples shown in Table 4-4.

Both products were pasteurized for panelist food safety and because the commercial products had undergone thermal treatment. After pasteurization at 90°C for 30s, the customer preference test on pilot plant scale products was performed to investigate the suitability of MFE as a sweetener in orange juice beverages. Comparison between stevia and MFE in terms of sweetness, freshness and aftertaste and other attributors were also determined in orange juice products.

A total of 92 panelists evaluated the tested samples. The behavioral question suggested that more than 80% of panelists consume light/low-calorie beverages (such as diet soda, flavored water, etc.) at least 2-3 times a month. Additionally, more than 70% panelists showed that they consume light/low-calorie juice beverages several times a month.

Results of overall liking scores suggested that when tasted three samples in randomize order, panelists preferred samples with MFE sweetener over the commercial stevia-sweetened product ($P \leq 0.05$; Table 4-5). The best overall liking was received in sample B made with 25% less MFE regarding to sample A. Trop50® made with stevia, by contrast, received the lowest overall liking score. More specifically, panelists preferred sample B for its appearance and flavor (Table 4-6), indicating that a lower concentration of MFE sweetener in product resulting a proper balance that was more acceptable compared with equivalent sweetness blends. Kamerud and others (2007) also found that perceived sweetness didn't contribute significantly to overall liking, which means panelists who rated a product with sweetener high in sweetness were not always more likely to rate it high for liking. In contrast, the average bitterness rating for a compound was found highly correlated with liking, and the perceived bitterness was obviously increased as the concentration of a given sweetener increasing.

According to the results of overall appearance rating, Trop50® was evaluated with the highest score (Table 4-2). However, more than 25% of panelists thought the color of Trop50® was "too dark", which was consistent with the result of instrumental color analysis. Also, it might suggest that people's preferences for appearance could increase with the darkness. Freely comment result supported the preference test results that orange juice with light color was at least equally acceptable for panelists to samples with dark color. For other major tastes, 60% to 68% of the panelists thought B samples were "Just-about-right" for sweet (68%), sourness (60%) and bitterness taste (67%; $P \leq 0.05$; Table 3), which was significantly higher than the percentages of other tested samples. Trop50® received a higher rating in "too bitter" selection. Similar result was obtained by Freitas, Dutra and Bolini (2016), stevia showed a higher perception of bitter taste and the maximum intensity persisted longer than sucrose and other tested sugar substitutes: sucralose, aspartame, Neotame and saccharin. This indicates that the addition of color additive made a difference in the acceptability of prototype OJB. Most interestingly, in addition to the influence on taste, the texture was rated no significant difference among samples, even though the analytical viscosity of sample A and B were nearly half lower than that of Trop50®. This evidence confirms that products with proper color and appearance could increase customers' acceptance on mouthfeel. Koza et al. (2005) found that a food's color could influence flavor perception by modifying the orthonasal and retronasal odor intensities. Also, the somatosenses of the food and overall multisensory flavor percept could be influenced by color (Spence et al., 2010). Their results were consistent with the result of this study as well. The comparison of sample A and preliminary formulated sample indicated that the color plays a significant role in customers' overall flavor perception on diluted orange juice beverages.

The results of "check all that apply" questions (Fig. 4-6) showed that 63% of customers selected "Smooth", and 45% of them used "Refreshing" to describe sample B. As for Trop50®, 51% panelists thought it was "Artificial" and 29% thought it was "Chemical", which are both the highest negative rating among tested samples. Descriptor analysis suggested that MFE sweetener has a fresh and natural flavor note in prototype orange juice beverage, compare to stevia which may contribute to some negative and bitter taste. This also confirmed the result of aftertaste evaluation in preliminary test.

In the end, consumers indicated their purchase intent of each sample. The result indicated that more than 64% of panelists were willing to buy sample B, which was consistent with the ranking results that sample B was rated the best among samples.

Result also suggested that a favorable taste of products can increase people's purchase intent. Finally, results also indicated that healthier and sugar reduction label claims can increase customers' purchase intent of orange juice beverages significantly ($P \leq 0.05$). The top two boxes rate increased to 73% when product information "all-Natural Light Orange Juice Beverage, made with 50% less sugar and calories than regular juice, no artificial sweeteners." was given, consistent with Krutulyte and others (2011) reported that familiarity of products and functional ingredients leads to higher purchase intent for functional foods. Additionally, results from Reis and others (2017) suggested that the information increased consumer sensory and hedonic rating of sugar-reduced products, and it could influence consumer perception of physical health and emotional aspects of wellbeing.

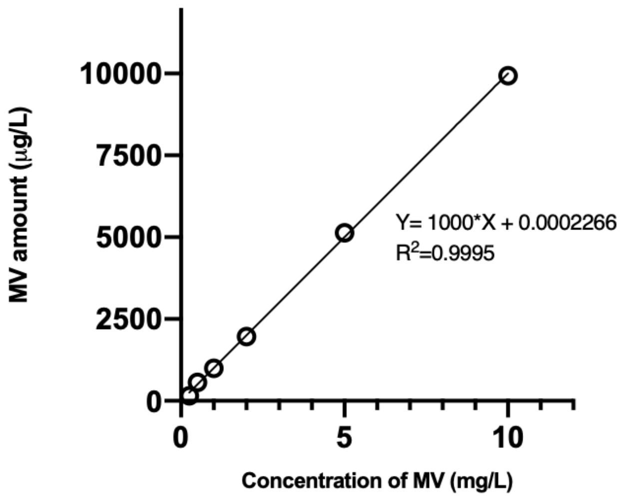


Figure 4-1. The calibration curve of the dependence of a peak area on the concentration of mogroside V:MV, mogroside V.

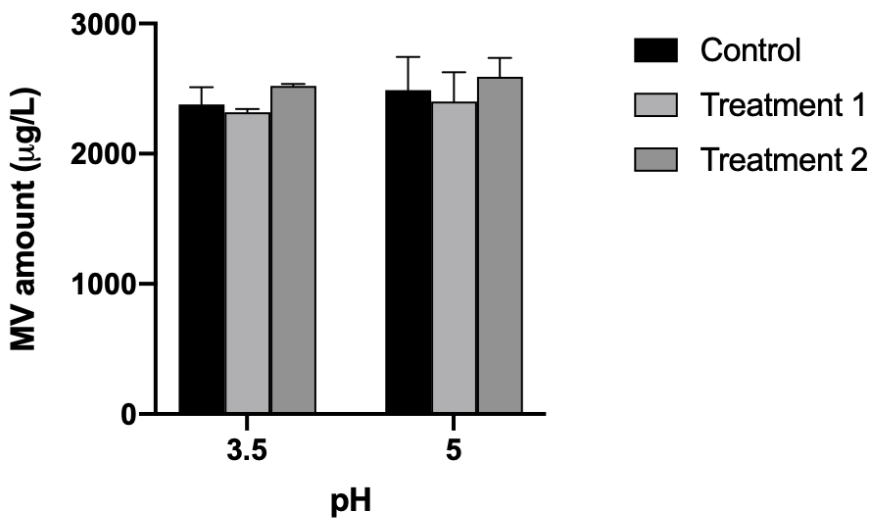


Figure 4-2. Effect of heat treatment1(72°C, 15s) and treatment 2 (90°C, 30s) on mogroside V in pH 3.5 and pH 5.0 model liquids.

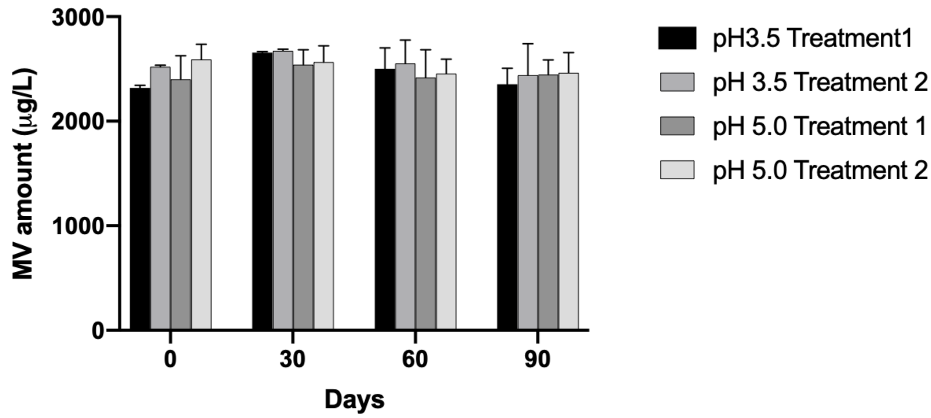


Figure 4-3. Effect of storage on mogroside V in pH 3.5 and pH 5.0 model liquids after heat treatment1(72°C, 15s) and treatment 2 (90°C, 30s).

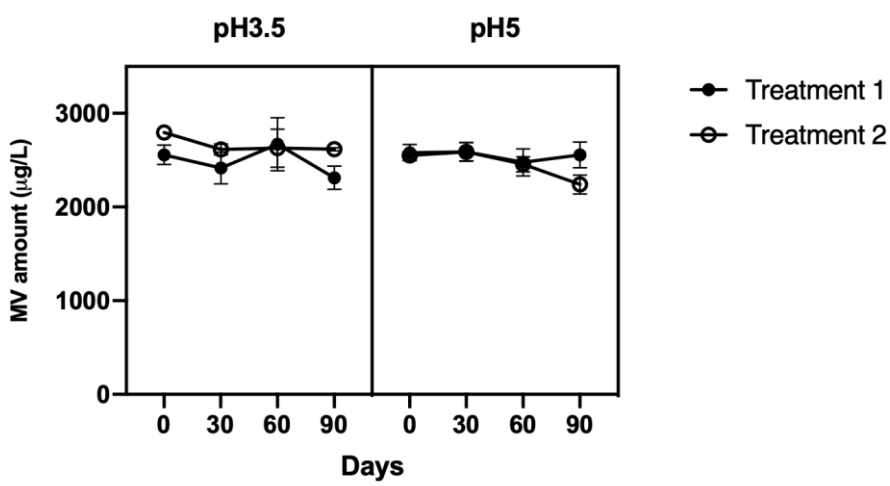


Figure 4-4. Results from repeated storage test: effect of storage on mogroside V in heat treated (treatment 1,72°C, 15s; treatment 2, 90°C, 30s) model systems.

Table 4-1. pH, soluble solids and acidity of three orange juice products

Parameters	Tropicana Orange Juice[a]	Trop50® Orange Juice Beverage	Formulated Orange Juice Beverage
pH	3.78	3.88	3.52
°Brix (%, w/w)	11.6	6.0	7.8
Acidity (g/L)	0.80	0.68	0.63

[a] Results represent mean values from triplicate analyses.

Table 4-2. Descriptive analysis scores for attributes of appearance and overall flavor in preliminary test evaluated by 31 panelists

Products	Appearance[a,b]	Color	Aroma	Flavor	Freshness
Tropicana Orange Juice	6.8a	6.7a	6.7a	6.6a	6.8a
Trop50® Orange Juice Beverage	6.4a	6.2a	5.9b	5b	4.8c
Formulated Orange Juice Beverage	5.6b	5.4b	6.1b	5.3b	5.5b

[a] Evaluation of each attribute was based on 9-hedonic scale (1=Dislike extremely, 5=Neither like nor dislike, 9=Like extremely). [b] Different lowercase letters indicated a significant difference.

Table 4-3. Descriptive analysis on attributes of basic flavor and texture in preliminary test evaluated by 31 panelists

Products	Sweetness[a,b]	Sourness	Thickness	Tartness
Tropicana Orange Juice	2.8b	3.1a	2.9a	3.2a
Trop50® Orange Juice Beverage	3.1b	2.7b	2.8a	2.9a
Formulated Orange Juice Beverage	3.7a	2.6b	2.5b	2.5b

[a] Evaluation of each attribute was based on Just-about-right scale 5-anchored hedonic scale (e.g. 1= Not nearly sweet enough, 2=Somewhat not sweet enough, 3=Just about right, 4=Somewhat too sweet, 5=Much too sweet). [b] Different lowercase letters indicated a significant difference.

Table 4-4. Quality evaluation of Trop50® and reformulated samples A and B

Parameters	Trop50®	Sample A	Sample B
pH	3.88	3.45	3.45
Brix(%, w/w)	6.0	7.6	7.1
Color[a]	L*=24.54 a*=13.33 b*=41.76	L*=32.24 a*=9.73 b*=53.24	L*=32.22 a*=9.76 b*=53.34
Viscosity (cp) [b]	23.0	13.2	13.1

[a] Color scores shown in CIELAB color scale
[b] Rotational viscometer: 100RPM, S01spindle

Table 4-5. The overall liking and overall appearance liking results for samples in final sensory evaluation with 92 panelists

Samples	Overall Liking[a,b]	Overall Appearance	Overall Texture
A	5.72 ab	6.54 b	6.08a
B	6.16 a	6.70 ab	6.22a
Trop50®	5.63 b	6.92 a	6.27a

[a] Attributes were scored on a 9-point hedonic scale where dislike extremely = 1 and like extremely = 9.
[b] Different letters in rows following means of each attribute indicate significant differences (P ≤ 0.05).

Table 4-6. Customer preference results for low-calorie orange juice beverages in final sensory evaluation with 92 panelists

Samples	Color[a,b,c]	Sweetness	Sourness	Bitterness
A	65.22% b	48.91% c	48.91% b	54.35% b
B	70.65% a	68.48% a	59.78% a	67.39% a
Trop50®	70.65% a	58.70% b	53.26% ab	52.17% b

[a] Just-about-right scales were scored on a 5-point scale
[b] Percentage of consumers that selected “Just-about-right” options is presented.
[c] Different letters in rows following means of each attribute indicate significant differences (P ≤ 0.05).

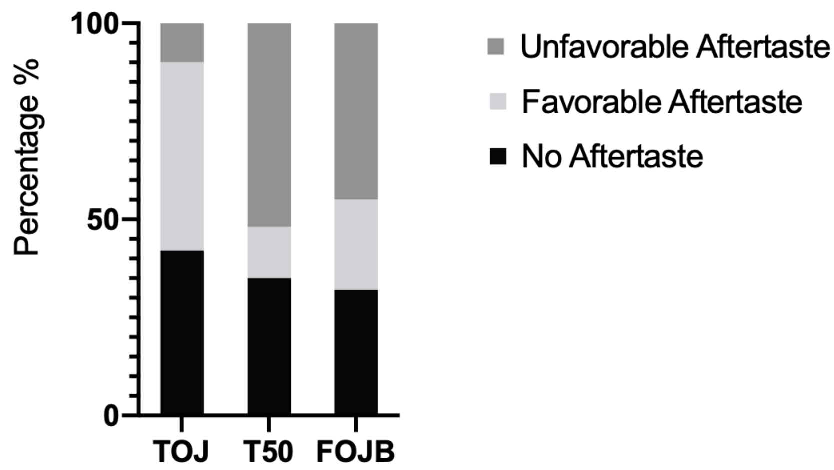


Figure 4-5. Result of aftertaste evaluations on tested beverages in preliminary test with 31 panelists: TOJ, Tropicana Orange Juice; T50, Trop50®; FOJB, Formulated orange juice beverage.

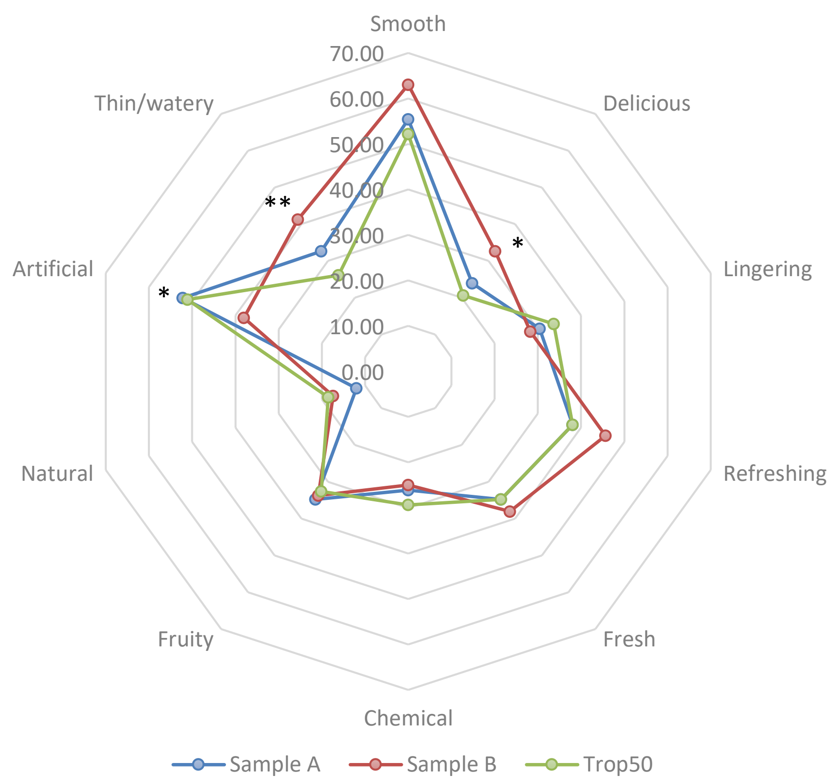


Figure 4-6. Results of descriptive evaluations on Trop50® and prototype samples in final sensory evaluation with 92 panelists: *significant difference (P<0.05); ** (P<0.1).

CHAPTER 5
CONCLUSION

The stability and sweetness intensity perception of MFE in orange juice beverages, and the appropriate sweetness concentration levels in orange juice beverages for target customers were determined in this study. Citric acidified aqueous solutions under two pH levels (pH 3.5 and 5.0) with 25mg/100mL mogroside V were stable after different heat treatment regimes and during shelf-life storage over 90 days. Results also show that this compound didn't have any interaction with other ingredients in the samples during storage. The low-calorie orange juice beverage sweetened partially by commercially available MFE was acceptable by target panelists. Lowing the sweetness concentration by 25% of MFE in Orange juice beverage samples increased overall liking significantly. People preferred MFE as a sweetener in sugar-reduced orange juice beverages over than stevia. In conclusion, MFE can be considered as a potential sugar substitute in the development of a new low-calorie orange juice beverages.

Possible limitation of this study would be that all the panelists were recruited from Gainesville, FL area, which may not represent the entire US population. In addition, the orange juice used in final formulation was commercial pasteurized product, which means these orange juice in final samples had been pasteurized twice. However, that would likely be the case for the commercial product as well, as NFC juice is usually pasteurized before storage and subsequent use in formulated products. Pasteurization times may also influence the freshness or other flavor attributes in beverages. Further research is needed to better understand the impact of heat treatment on orange juice beverages and sweetener. Different flavoring agents and formulations of Trop50® may also affect consumer acceptance. Further research could evaluate the different varieties of MFE and the blends of different ingredients in orange juice beverages and other food products.

APPENDIX A
INGREDIENT LIST OF TROP50®

The ingredients found from the package of Trop50® as follows: Filtered water, not from concentrate pasteurized orange juice, orange juice concentrate, potassium citrate, malic acid, citric acid, magnesium phosphate, ascorbic acid (Vitamin C), calcium citrate, beta-carotene, purified stevia leaf extract, tocopherols (vitamin E), gellan gum, natural flavor, niacinamide (vitamin B3), thiamin hydrochloride (vitamin B2), pyridoxine hydrochloride (vitamin B6), riboflavin (vitamin B2).

APPENDIX B
FORMULATION DETAILS

Table B-1. The formula information of model juice in stability test

Samples	Water (mL)	Citric Acid (g)	Sodium Citrate (g)	Sucrose (g)	Mogroside V (mg)
Model Juice (pH 3.5)	1000	3.022	1.255	60	25
Model Juice (pH 5.0)	1000	1.625	3.393	60	25

Table B-2. The formula information of samples in sensory tests

Samples	Commercial Orange Juice (mL)	Water (mL)	Citric Acid (g)	Commercial MFE (g)	Color Additive (g)
Preliminary Sample	500	500	2.25	16	--[a]
Final Formula A	500	500	2.25	16	1.5g “Spicy Yellow” and 0.14g “Mandarin”
Final Formula B	500	500	2.25	12	1.5g “Spicy Yellow” and 0.14g “Mandarin”

[a] -- indicates the ingredient was not used in the formula

APPENDIX C
FINAL SENSORY EVALUATION BALLOT

Welcome!

Today's Samples: Orange Juice Beverages

Please indicate your gender:
___Female
___Male

Please indicate your age:

In which of the following groups would you most likely place yourself?
___Caucasian
___Hispanic/Latino
___African-American
___Native American/American Indian
___Asian
___Other

How often do you consume orange juice?
____Daily
____3-4 times a week
____Once a week
____2-3 times a month
____Monthly
____I like orange juice, but I don't drink it often

How often do you consume light/low-calorie beverages? For example, diet soda.
____Daily
____2-3 times a week
____Once a week
____2-3 times a month
____Monthly
____Rarely

How often do you consume light/low-calorie **juice beverages**?
____Daily
____2-3 times a week
____Once a week
____2-3 times a month
____Monthly
____I like light juices, but I don't drink them often
____I don't like light juice beverages

The next several questions will be on the APPEARANCE of sample 602.

Please do NOT TASTE until you are instructed to do so.

Please indicate how much you like the **appearance** of sample 602.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

Please rate the **color** of sample 602.

Much too light	Somewh at too light	Just About Right	Somewh at too dark	Much too dark
1	2	3	4	5

Take a bite of cracker and a sip of water to rinse your mouth.
Remember to do this before you taste each sample.
You are now ready to taste sample 602.

Please indicate how much you like sample 602 **overall**.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

Please indicate how much you like the **flavor** of sample 602.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

Please indicate how much you like the **texture** of sample 602.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
1	2	3	4	5	6	7	8	9

How would you describe the **Thickness** for sample 602?
Thickness

Much too thin	Somewhat too thin	Just About Right	Somewhat too thick	Much Too thick
1	2	3	4	5

How would you describe the **sweetness** for sample 602?
Sweetness

Not Nearly Sweet Enough	Not Quite Sweet Enough	Just About Right	A Little Too Sweet	Much Too Sweet
1	2	3	4	5

How would you describe the **sourness** for sample 602?
Sourness

Not Nearly Sour Enough	Not Quite Sour Enough	Just About Right	A Little Too Sour	Much Too Sour
1	2	3	4	5

How would you describe the **bitterness** for sample 602?
Bitterness

Not Nearly Bitter Enough	Not Quite Bitter Enough	Just About Right	A Little Too Bitter	Much Too Bitter
1	2	3	4	5

Which of the following words best describes this light orange juice sample?
Smooth
Lingering
Delicious
Chemical
Refreshing
Fruity
Thin/Watery
Natural
Artificial

Other_____

How else would you describe sample 602?

Now that you have tasted sample 602, how likely would you be to PURCHASE it if it were available at a store where you usually shopped?

Definitely would not buy	Probably would not buy	Might or might not buy	Probably would Buy	Definitely would buy
1	2	3	4	5

Beginning with the sample on the left, please taste each sample again and RANK them from the MOST preferred to LEAST preferred.

Please carefully read the following product description and then answer the question below.
Introducing a NEW, all-Natural Light Orange juice, made with 50% less sugar and calories than regular juice, No artificial sweeteners, and only 50 calories per serving. Based on the product description above, how likely would you be to purchase the type of light or low-calorie orange juice beverages. If it were available at a store where you usually shop?

Definitely would not buy	Probably would not buy	Might or might not buy	Probably would Buy	Definitely would buy
1	2	3	4	5

Please feel free to comment on what you liked/dislike about sample 602.

____

____

____

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BIOGRAPHICAL SKETCH

Zhou Zou came from Changsha, the capital of Hunan province in the south part of China. Her hometown is known for its long history, creative and passionate people, traditional street food and the largest entertainment television media system in China. Changsha gave her a lot of creativity and imagination, as well as the courage to try new things. Zhou grew up with her grandparents, who has influenced her a lot. Her grandfather is a hardworking and popular doctor who has no judgement of his patients and received many respects and awards. Zhou developed an early interest in life science during her childhood. At meantime, her grandmother, who is still the best chef in her mind, always has tons of ideas for delicious homemade food. Food and love are tightly tied through Zhou's life. Her parents have always insisted on giving her the best education and developing her independency and critical thinking ability. Zhou and her family love travelling, these interesting experiences with different cultures inspired her to study aboard. During her undergraduate studies in Nanjing Agricultural University, Zhou completed her course works in the first three years and was admitted as an exchange student in University of Florida. After obtaining her bachelor's degree from China, she worked as a master's student under Dr. Renée Goodrich-Schneider with a specialization in low-calorie orange juice beverage development. Under her instruction, Zhou gained the experiences in working as a lab assistant, getting food safety training, presenting at conferences, participating competition and receiving awards. Upon graduation, Zhou plans to pursue a career to apply her skills in food industry.